AWARD NUMBER: W81XWH-14-1-0473

TITLE: Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS

PRINCIPAL INVESTIGATOR: EUN-SIL HWANG

CONTRACTING ORGANIZATION: DUKE UNIVERSITY
Durham, NC 27708

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# 1. REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| October 2015 | Annual | 30 Sep 2014 - 29 Sep 2015 |

**4. TITLE AND SUBTITLE**

Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-14-1-0473

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

EUN-SIL HWANG

E-Mail: shelley.hwang@dm.duke.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Duke University
2200 W Main St Ste 710
Durham NC 27708-4677

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The project is designed to test whether genetic and/or tumor environmental heterogeneity is a driving force in progression of breast DCIS. Our project, a collaboration between Duke and ASU, has made substantial progress on all 4 aims of the original proposal and is on track to meet 24 month milestones. Primary achievements during the first year are: 1) Case and control identification through extensive database and medical record searching at Duke, 2) Development of methods for isolating DNA from archival DCIS lesions, 3) Deep and comprehensive full exome sequencing from 20ng of DNA isolated from these archival specimens, 4) Comparison of analytic methods to characterize somatic mutations from this full exome sequencing, 5) Application of sequencing library DNA to Illumina SNP arrays for copy number assessment, 6) Development of dual immune-staining on DCIS lesions using 7 pairs of antibodies, 7) Sharing of images from these stains with collaborators for quantitative analysis, 8) Identification of a series of upstaged DCIS cases for the radiology aim, 9) Development of image analysis methods for digital mammograms, 10) Approval of TBCRC protocol for the validation aim to initiate collection of DCIS that either did or did not progress to invasive cancer, 11) Full integration of team members over the past year via frequent conferencing, face to face meetings, and constant communication. This multi-disciplinary progress puts our group into an ideal position to fully implement the aims of the project and reach our intermediary and ultimate goals.

**15. SUBJECT TERMS**

DCIS, intra-tumor heterogeneity, genetic diversity, phenotypic diversity, somatic evolution, microenvironment, mammographic biomarkers

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| U | U | U | UU | 22 | 19b. TELEPHONE NUMBER *(include area code)* |

## Table of Contents

## 1. INTRODUCTION

Ductal carcinoma in situ (DCIS) of the breast is an increasingly common diagnosis that is related to aggressive screening patterns (mammography). This "pre-invasive" lesion may progress to invasive cancer, but does so at a relatively low frequency. Nonetheless, it is commonly treated with extensive surgery, radiation, and hormonal therapy even though most of these lesions would never progress to invasive cancer. Thus, there is a pressing clinical need to stratify the risk of DCIS tumors into those in need of intervention and those that can be safely monitored without intervention. Our project is designed to address this need by characterizing the evolvability of DCIS, detecting those that have a high likelihood of evolving to malignancy versus those that are likely to remain indolent.

## 2. KEYWORDS

DCIS, cancer progression, intra-tumor heterogeneity, genetic diversity, phenotypic diversity, somatic evolution, microenvironment, mammographic biomarkers

## 3. ACCOMPLISHMENTS

### What were the major goals of the project?

*Aim 1. Determine whether genetic diversity of DCIS is greater in DCIS with adjacent invasive disease compared to DCIS without progression.* Diversity measures must be derived from geographically distinct areas of tumor. Genetic divergence of the DCIS component of tumors will be measured based on exome sequencing and SNP arrays run on two separate regions of the tumor, as well as normal tissue, in patients with DCIS either with or without invasion to determine the association between genetic diversity and progression to malignancy. Genetic diversity will be measured by the genetic divergence between the tumor samples, that is, the proportion of the genome that differs between the two samples from the same tumor.

*24 Month Milestones:*

- Protocol preparation, IRB submission and approval: Complete (Duke eIRB Pro00054515, initial Duke approval, 5/27/2014 and renewed for the current year), DOD IRB approval in place

- Case identification and tissue block selection: Through a variety of available databases, we have identified a large number of potential cases and controls with tissue available in the Duke Pathology archives. Each potential case and control requires extensive chart and pathology review in order to determine final eligibility and usability. We are now performing these reviews with newly created case report forms and databases to capture the information.

- Sectioning and coring of tissue blocks: New sections from candidate paraffin blocks are made, stained with H&E, reviewed by the study pathologist, and these slides are scanned for analytic and archival purposes. Additional slides from useful blocks (containing a

sufficient amount of the DCIS lesion of interest) are obtained and macro-dissected for DNA extraction. Additional sections (every other one) are also stored for immunohistochemical (IHC) analysis of key measures of tumor and micro-environmental heterogeneity. This process has been fully implemented and we are moving through both cases and controls in this manner.

- DNA extraction of test cases: **complete**.

- Exome sequencing of test cases: we have investigated a number of platforms and collaborators for the DNA sequencing and SNP analysis. Since we are working with small amounts of FFPE DNA, standard methodologies do not readily apply. Based on a pilot set of 14 DNA samples, we have settled on the Genome Center at Washington University run by Elaine Mardis. Dr. Mardis is working with us closely and her group has developed cutting-edge methods for producing high quality data from these specimens. In addition to full-exome capture, the method employs additional enrichment for a panel of 83 genes to ensure high coverage of the most commonly altered breast cancer driver genes. Over the past several months, Wash U. sequenced 20ng from 14 individual DNA samples derived from 4 subjects (germ line sample plus 2 DCIS containing samples with two duplicates=14) and returned the data to us for analysis. In addition, we also asked the Wash U. group to perform a basic analysis of the data for comparison to our informatics pipeline. Most important, they were able to derive interpretable sequence data from 20ng of FFPE DNA with average coverage ranging from 10-80X. Our collaborators, Carlo Maley and Trevor Graham, analyzed these data and found numerous candidate mutations with estimated allele frequencies. The Wash U. group recently returned their analysis and we are now in the process of comparing the results.

Below are tabular results from the first 14 samples indicating the number of reads indicated by the size of the BAM file and the duplicate rate. Case#31 had the highest duplicate rates which is consistent with it having the lowest quality of DNA assessed by various QC metrics. However, even the sample with 82% duplicate rate yielded interpretable data.

| Block ID | Case# | Duplicate | Germline | raw bam size (Gb) | after rmdup (Gb) | dup rate |
|---|---|---|---|---|---|---|
| LK38-020-node-A1 | 20 | | x | 4.4 | 3 | 0.32 |
| LK40-020-B3 | 20 | 1 | | 4.3 | 2.6 | 0.40 |
| LK39-020-B6 | 20 | | | 8.2 | 5.9 | 0.28 |
| LK-B-40-020-B3 | 20 | 1 | | 4.3 | 2.4 | 0.44 |
| LK33-028-K12 | 28 | 2 | | 3.9 | 3 | 0.23 |
| LK32-028-node-AF1 | 28 | | x | 4 | 3 | 0.25 |
| LK33-028-K12 | 28 | 2 | | 4.5 | 3.6 | 0.20 |
| LK34-028-K18 | 28 | | | 4.3 | 3.3 | 0.23 |
| LK42-029-D8 | 29 | | | 3.3 | 2.6 | 0.21 |
| LK43-029-node-A2 | 29 | | x | 2.4 | 2.1 | 0.13 |
| LK41-029-D5 | 29 | | | 3.2 | 2.7 | 0.16 |
| LK48-031-A34-2 | 31 | | | 4.4 | 1.8 | 0.59 |
| LK47-031-A21-2 | 31 | | | 3.3 | 0.6 | 0.82 |
| LK49-031-node-D2 | 31 | | x | 3.6 | 1.9 | 0.47 |

- SNP arrays: In order to better estimate copy number variation (CNV), we are also analyzing DNA from the two areas of DCIS from each case using high density single nucleotide polymorphism (SNP) arrays. We are using the human Omni 2.5 array from Illumina to accomplish this aspect of the project. Since DNA from the primary samples is limiting (macrodissected DCIS), we have been testing whether sequencing libraries generated for exome sequencing can be directly applied to these arrays. Data on the first batch of these samples using this approach will be available for analysis within the next two months.

*Aim 2. Determine whether phenotypic diversity of DCIS and the tumor microenvironment (TME) is greater in DCIS with adjacent IDC compared to DCIS without IDC.* Since genomics is not the sole driver of tumor behavior, we will phenotypically characterize DCIS and its microenvironment including markers of hypoxia, migration, proliferation, matrix organization, and immune signaling in the same samples used in Aim 1. We will employ automated image analysis to compute microenvironmental divergence to determine if specific components of the TME, or the divergence between TMEs from the same tumor, differs between DCIS with and DCIS without adjacent IDC.

We have brought a new collaborator into the team, Dr. Yinyin Yuan from the Center for Evolution and Cancer at the Institute for Cancer Research in London. Dr. Yuan is an expert in computational image analysis of histological sections of breast cancer, and the application of ecological and other spatial statistics to those images [1-4]. She and her group will provide quantitative analyses of immunohistochemical stained sections to evaluate tumor heterogeneity.

*24 Month Milestones:*

- IHC staining of candidate markers (test cases): We have obtained and characterized a series of antibodies representing our initial targets including ER, PR, KI-67, COL15A1, RHOA, RAC, CA9, HIF1a, FOXP3, and cleaved Caspase 3. We have piloted dual staining for sets of these antibodies on test cases of breast cancer and will soon be staining for these antigens on DCIS cases and controls. Dual staining conditions will be

optimized in collaboration with Dr. Yinyin Yuan's lab who will be doing the automated, quantitative scoring and analysis of the stained tissues.

- Scan IHC results for Automated image analysis (AIA): Not started yet.

- Automated image analysis (AIA) of tumor and stromal markers of heterogeneity: Dr. Yuan's team is adapting their algorithms for dual staining. They already have successfully analyzed both clustering of cell types [2, 3], and co-localization (interleaving) among different cell types (manuscript under review).

*Aim 3. Create and test a computational learning algorithm to compare mammographic characteristics and diversity measures in pure DCIS compared to DCIS with IDC.* A weighted computational algorithm using mammographic features of lesional and stromal characteristics as well as heterogeneity measures derived from Aims 1 and 2 will be constructed. The tool will be designed to allow for radiologic discrimination between good and poor prognosis DCIS, and will be evaluated in a validation set.

*24 Month Milestones:*

- This work is being performed at Duke by our radiology collaborators, Joseph Lo and his group. The first steps are to define permissible values for each input class: For automated identification of lesions representing DCIS on mammography, we created preliminary algorithms for the detection, segmentation, and clustering of microcalcifications. The multi-step process is based upon median filtering and global as well as local thresholding, with several false positive rejection steps using clustering and morphology rules. Using images from 12 randomly selected subjects, we performed a grid search to optimize initial algorithm parameters. We are in the process of implementing initial algorithms to automatically extract imaging features from the resulting microcalcification clusters. We have also developed graphical user interfaces to facilitate radiologists providing ground truth for lesion size and location. We currently have preliminary but fully functional algorithms for both cluster identification and feature extraction.

- Identify test set and validation set: We are identifying the cohort of subjects to be used for the main study. Based on our inclusion and exclusion criteria, we have conducted several searches into our electronic medical records to identify qualifying DCIS cases from our institution. From over 1300 initial candidates, we have so far identified 161 potential subjects, from which we will verify availability of imaging and other required clinical data.

*Aim 4. Test the predictive performance of the best diversity measures in an independent validation set of pure DCIS with and without subsequent invasive recurrence.* Genotypic and phenotypic measures of diversity derived from Aims 1-2 will be applied to an independent case-control, longitudinal, tissue bank of DCIS with and without invasive recurrence to validate their utility.

*24 Month Milestones:* This aim will be carried out after aims 1-3 are complete. However, we have already obtained approval of our protocol to obtain these specimens through the Translational Breast Cancer Research Consortium (TBCRC). Through oral and written presentations to the TBCRC, we have identified 11 high volume academic medical center members of the consortium who have expressed interest in participating. Once the protocol is approved at Duke, we will circulate it to these 11 outside sites for them to obtain regulatory approval. We anticipate beginning to accrue these specimens (and attendant data) within the next 6 months.

**What was accomplished under these goals?**

Our primary goals have been met including, most importantly, identifying the most efficient method of sequence generation from small amounts of fixed DNA. Further, based on our databases, we are confident of accruing sufficient cases and controls at Duke to fulfill the goals of the project. Overall, we are in excellent position to complete the proposed work in the project period along the time line that was provided.

**What opportunities for training and professional development has the project provided?**

We hired a new post-doctoral fellow (Lorraine King) to oversee the work at Duke and coordinate the activities at our other participating sites. Dr. King is acquiring new skills and developing existing ones as part of this complex effort.

**How were the results disseminated to communities of interest?**

We are reporting the early sequencing results at the San Antonio Breast Cancer Symposium in December 2015.

**What do you plan to do during the next reporting period to accomplish the goals?**

Aim 1: We will continue to identify potential cases and controls through Duke Pathology archives and databases and carefully examine each subject for their eligibility. Diagnostic slides from candidate subjects will be evaluated by our study pathologist to determine if there is sufficient material to work with and ones that pass this metric will be included in the study. New unstained slides will be ordered from these cases for macrodissection and immunohistochemical staining. DNA extracted from these slides will be exome sequenced and applied to high density SNP arrays. Returned data from these assays will be analyzed for genetic heterogeneity compared to germ line DNA (isolated from normal lymph nodes).

Aim 2: We will begin to analyze cases and controls using a series of antibody stains described in the proposal. Scanned images of these stained slides will be provided to Dr. Yuan for image analysis and quantification. Dr. Yuan's team will adapt their algorithms to quantify dual stained slides. Heterogeneity of expression of these protein markers associated with the tumor, basement membrane, vasculature, and immune infiltrate will be incorporated into measures of genetic heterogeneity.

Aim 3: We anticipate creating a preliminary database of at least 50 cases, which will be sufficient to drive the continued development of the mammography lesion identification and feature extraction algorithms. These images will be analyzed with the algorithms to derive measures of tumor heterogeneity as per the original goals of the proposal.

Aim 4: The TBCRC protocol was recently approved and is now being evaluated by the Duke IRB. Eleven (12 including Duke) will begin to contribute cases and controls to this validation aim during the coming budget year.

## 4. IMPACT

Successful completion of this project will lead to a variety of biomarkers (genetic, IHC and radiographic) to distinguish high risk from low risk DCIS. This would reduce patient suffering and conserve clinical resources for the women with low risk DCIS, and focus management efforts and clinical resources on women with high risk disease, potentially justifying the risks of interventions. As the project is in its initial stages, these important impacts await in the future.

### What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

### What was the impact on other disciplines?

Nothing to report.

### What was the impact on technology transfer?

Nothing to report.

### What was the impact on society beyond science and technology?

Nothing to report.

## 5. CHANGES/PROBLEMS

### Changes in approach and reasons for change

There have been no changes in approach.

### Actual or anticipated problems or delays and actions or plans to resolve them

So far the problems that have emerged have been primarily technical. Full exome sequencing from small amounts of FFPE tissue is at the limit of current technical practice. Further, analyzing these data is also a challenge. We are now confident in our ability to generate high coverage and high depth sequencing data from as little as 20ng of FFPE DNA. We are also performing technical replicates to determine the reproducibility and noise that is in the system.

These data are now being analyzed and will guide the eventual analytic paradigm that will be used going forward.

In order to evaluate heterogeneity within a tumor, we require that there are as few normal cells as possible in the extraction. We initially evaluated laser capture microdissection and found that the DNA yields were insufficient to acquire comprehensive sequence data. Therefore, in conjunction with our study pathologist, we are now routinely marking slides for **macro**dissection which provides sufficient DNA and excellent purity.

We are currently developing our automated imaging analyses of dual stained tissue sections with Dr. Yuan. Dual staining is challenging in and of itself, because one must find staining conditions that work well for both antibodies. We anticipate that there may be difficulties distinguishing the two colors in the same pixel when a cell is positive for both markers, but this has yet to be tested. If that proves insurmountable, we will pair a nuclear stain with a cytoplasmic stain so that they do not overlap.

### Changes that had a significant impact on expenditures

None

### Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None

### Significant changes in use or care of human subjects

None

### Significant changes in use or care of vertebrate animals.

Not applicable.

### Significant changes in use of biohazards and/or select agents

Not applicable

## 6. PRODUCTS

### Publications


1. Walther, V., Hiley, C.T., Shibata, D., Swanton, C., Turner, P.E., and **Maley, C.C.**: Can oncology recapitulate paleontology? Lessons from species extinctions. Nature Reviews Clinical Oncology, 12:273-285, 2015. doi:10.1038/nrclinonc.2015.12 Published. Acknowledged federal support.
2. Caulin, A.F., **Maley, C.C.:** Solutions to Peto's Paradox Revealed by Mathematical Modeling and Cross-Species Cancer Gene Analysis. Philosophical Transactions of the

Royal Society of London B, 370 (1673):20140222. Published. Acknowledged federal support.

3. Aktipis, C.A., Boddy, A.M., Jansen, G., Hibner, U., Hochberg, M.E., **Maley, C.C.**, Wilkinson, G.S.: Cancer across the tree of life: Cooperation and cheating in multicellularity. Philosophical Transactions of the Royal Society of London B, 370 (1673):20140219. Published. Acknowledged federal support.
4. Noemi Andor, Trevor A. Graham, Marnix Jansen, Li C. Xia, C. Athena Aktipis, Claudia Petritsch, Hanlee P. Ji, Carlo C. Maley: Pan-cancer analysis of the extent and consequences of intra-tumor heterogeneity. Under review at Nature Medicine. Acknowledged federal support.
5. Carlo C. Maley, Konrad Koelble, Rachael Natrajan, Athena Aktipis and Yinyin Yuan: An ecological measure of immune-cancer colocalization as a prognostic factor for breast cancer. Under review at Breast Cancer Research. Acknowledged federal support.

### Website(s) or other Internet site(s)

None

### Technologies or techniques

Nothing to report

### Inventions, patent applications, and/or licenses

Nothing to report

### Other Products

Case report forms for Duke and outside cases and databases to efficiently capture this information

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

Co-PI: Dr. Shelley Hwang (M.D., M.P.H.): Duke University (no change)
Co-PI: Dr. Carlo C. Maley (Ph.D.): Arizona State University (no change)

Co-Investigators:
Dr. Jeffrey Marks (Ph.D.): Duke University (no change)
Dr. Joseph Geradts (M.D.): Duke University (no change)
Dr. Joseph Lo (Ph.D.): Duke University (no change)
Dr. Jay Baker (M.D.): Duke University (no change)
Dr. Trevor Graham (Ph.D.): Barts Cancer Institute, Queen Mary University of London (no change)
Dr. C. Athena Aktipis (Ph.D.): Arizona State University (no change)
Dr. Shane Jensen (Ph.D.): University of Pennsylvania (no change)

| | |
|---|---|
| **Name:** | Eun-Sil S. Hwang |
| **Project Role:** | PI |
| **Research Identifier (e.g. ORCID ID):** | eBRAP: HWANGSH |
| **Nearing person month worked:** | 2 |
| **Contribution to Project:** | Overall project supervision |
| **Funding Support:** | Susan G. Komen Breast Cancer Foundation, Patient-Centered Outcomes Research Institute, National Institutes of Health, University of California at San Francisco |

| | |
|---|---|
| **Name:** | Jeffrey R. Marks |
| **Project Role:** | Co Investigator |
| **Research Identifier (e.g. ORCID ID):** | orcid.org/0000-0002-2054-5468 |
| **Nearing person month worked:** | 2 |
| **Contribution to Project:** | Supervising laboratory component |
| **Funding Support:** | National Institutes of Health, Arizona State University |

| | |
|---|---|
| **Name:** | Jeffrey P. Groth |
| **Project Role:** | Lab Research Analyst |
| **Research Identifier (e.g. ORCID ID):** | N/A |
| **Nearing person month worked:** | 1 |
| **Contribution to Project:** | Histology support |
| **Funding Support:** | No longer participating in this project |

| | |
|---|---|
| **Name:** | Joseph Y. Lo |
| **Project Role:** | Co Investigator |
| **Research Identifier (e.g. ORCID ID):** | eRA Commons: JOLO123 |
| **Nearing person month worked:** | 1 |
| **Contribution to Project:** | Leading the radiology component (aim 3) |
| **Funding Support:** | National Institutes of Health |

| | |
|---|---|
| **Name:** | Jay A. Baker |
| **Project Role:** | Co Investigator |
| **Research Identifier (e.g. ORCID ID):** | eRA Commons: BAKER103 |
| **Nearing person month worked:** | 1 |
| **Contribution to Project:** | Radiology clinical collaborator |
| **Funding Support:** | National Institutes of Health |

| | |
|---|---|
| **Name:** | Allison Haberstoh Hall |
| **Project Role:** | Co Investigator |
| **Research Identifier (e.g. ORCID ID):** | orcid.org/0000-0002-0773-5726 |
| **Nearing person month worked:** | 1 |
| **Contribution to Project:** | Study pathologist – primary tissue evaluation |
| **Funding Support:** | National Institutes of Health |

| | |
|---|---|
| **Name:** | Lars Johannes L. Grimm |
| **Project Role:** | Co-investigator |
| **Research Identifier (e.g. ORCID ID):** | orcid.org/0000-0002-3865-3352 |
| **Nearing person month worked:** | 1 |
| **Contribution to Project:** | Radiology clinical collaborator |
| **Funding Support:** | N/A |

| | |
|---|---|
| **Name:** | Lorraine King |
| **Project Role:** | Post-doctoral fellow |
| **Research Identifier (e.g. ORCID ID):** | N/A |
| **Nearing person month worked:** | 10 |
| **Contribution to Project:** | Study coordinator |
| **Funding Support:** | N/A |

| | |
|---|---|
| **Name:** | Mengyu Wang |
| **Project Role:** | Post-doctoral fellow |
| **Research Identifier (e.g. ORCID ID):** | N/A |
| **Nearing person month worked:** | 5 |
| **Contribution to Project:** | Radiology image analysis |
| **Funding Support:** | No longer participating in this project |

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - Yes, please see the following updated Other Supports for Eun-sil S. Hwang, Jeffrey R. Marks, Joseph Y. Lo, Jay A. Baker and Allison Haberston Hall.

**HWANG, EUN-SIL S.**

**Changes to Active Support:**
The following grants listed on Dr. Hwang's previously submitted Active Support have ended: Habib's Society of Ambulatory Anesthesia grant entitled "Identification of women at risk for severe acute pain and persistent pain following breast surgery to identify women that are at risk for severe acute and persistent pain"; Hwang's SCRIPPS grant "12X5527".

The following grants are new to Dr. Hwang's Active Support: Hwang's PCORI grant entitled, "Comparing the Effectiveness of Guideline-Concordant Care to Active Surveillance for DCIS: an Observational Study"; Hwang's DoD grant entitled, "Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS"; Hwang's NIH grant, "1R01-CA185138-01"; Hwang's NIH grant, "5U10-CA180857-02"; Hwang's Subcontract with UCSD entitled, "PROMISE Komen Grant Sub with UCSF".

**Active**
**Title:** Immune and Collagen Basis of Breast Cancer
**Time Commitment:** 0.91 calendar months

**Role:** PI
**Sponsor:** Susan G. Komen Breast Cancer Foundation, KG110560 (Hwang)
**Contracting Grants Officer:** Pending
**Dates:** 11/30/11-11/29/15
**Funding:** $548,692
**Goals/Specific Aims:** The goals of this project are to accomplish the following:

1) Determine whether different cancer phenotypes are associated with BD, specific immune cell subtypes, and/or collagen status
2) Test whether specific immune profiles can be detected in the immune cell composition in circulating blood
3) Establish whether cancer progression from DCIS to invasive cancer is associated with specific immune signature and collagen structure

**Title:** Novel Immunotherapeutic Approach for Triple Negative Breast Cancer
**Time Commitment:** 1.2 calendar months
**Role:** PI
**Sponsor:** Susan G. Komen for the Cure, IIR12223053 (Hwang/Pruit)
**Contracting Grants Officer:** Pending
**Dates:** 10/30/12-10/29/16
**Funding:** $171,970
**Goals/Specific Aims:** The goal of this project is to develop a highly novel dendritic cell (DC)-based approach in which tumor associated antigen (TAA) RNA-transfected DC are co-transfected with RNA encoding soluble immune modulators that block immune-regulatory pathways that normally limit immune responses.

**Title:** Comparing the Effectiveness of Guideline-Concordant Care to Active Surveillance for DCIS: an Observational Study
**Time Commitment:** 2.4 calendar months
**Role:** PI
**Sponsor:** Patient-Centered Outcomes Research Institute (Hwang)
**Contracting Grants Officer:** Pending
**Dates:** 11/1/15-10/31/18
**Funding:** $486,658
**Goals/Specific Aims:**
**Aim 1:** Compare health related benefits and harms of treatment between patients electing GCC or AS for ductal carcinoma in situ (DCIS) in a large retrospective observational cohort
**Aim 2:** Evaluate the direct harms of treatment by comparing patient-reported outcomes (PRO) between patients electing GCC or AS for DCIS in a cross-sectional cohort at selected study sites
**Aim 3:** Synthesize data collected in Aims 1 and 2 to create a summary inventory of benefits and harms of all treatment options for DCIS in order to inform treatment decisions by patients and stakeholders.

**Title:** Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS
**Time Commitment:** 2.40 calendar months
**Role:** PI
**Sponsor:** Department of Defense, W81XWH-14-1-0473 (Hwang)

**Contracting Grants Officer:** Pending
**Dates:** 9/30/14-9/29/19
**Funding:** $1,482,598
**Goals/Specific Aims:** This proposal is based upon metapopulation and dispersal theories from ecology to predict cancer progression based on their effects on natural selection. This framework represents a novel approach that is a significant departure from traditional cancer biology, and could yield a universally applicable construct for understanding interactions between tumors and their environments.

**Title:** (PQC3) Genomic Diversity and Microenvironment as Drivers of Metastatis in DCIS
**Time Commitment:** 2.40 calendar months
**Role:** PI
**Sponsor:** National Institutes of Health, 1R01-CA185138-01 (Hwang)
**Contracting Grants Officer:** Suresh Mohla, 240-276-6220, mohlas@mail.nih.gov
**Dates:** 8/1/14-7/31/18
**Funding:** $337,027
**Goals/Specific Aims:** In this project, we propose to collect genomic, phenotypic, and radiographic measures of tumor cell diversity in DCIS and the tumor microenvironment, and test whether these diversity measures can identify which patients are most likely to develop metastatic disease. Deliverables from the proposal have high potential for rapid integration into clinical trials for active surveillance of DCIS, and in addition, could have universal relevance for management of other solid tumors.

**Title:** NCI National Clinical Trials Network U10
**Time Commitment:** 1.2 calendar months
**Role:** PI
**Sponsor:** National Institutes of Health, 5U10-CA180857-02 (Hwang/Crawford)
**Contracting Grants Officer:** Margaret Mooney, 240-276-6560, mooneym@mail.nih.gov
**Dates:** 4/1/14-2/28/19
**Funding:** $260,148
**Goals/Specific Aims:** The purpose of this grant is to designate multiple Principal Investigators for the Duke University NCI National Clinical Trials Network Lead Academic Participating Site application (RFA-CA-12-013) due to the involvement of Duke Faculty with numerous Cooperative Group Sponsors and the multiple modalities involved.

**Title:** PROMISE Komen Grant Sub with UCSF
**Time Commitment:** 0.91 calendar months
**Role:** PI
**Sponsor:** University of California-San Francisco
**Contracting Grants Officer:** Pending
**Dates:** 8/8/12-8/7/15
**Funding:** $79,724
**Goals/Specific Aims:** Data will be de-identified and entered into password-protected HIPAA-compliant database with access limited to those researchers directly participating on this project. Data will be used to adjust for factors associated with outcomes of interest and interactions between covariates.

**Jeffrey R. Marks, Ph.D.**

**Changes to Active Support:**
The following grants listed on Dr. Marks's previously submitted Active Support have ended: NIH grant entitled, "Epidemiology of Ovarian Cancer in African-American Women"; Dr. Marks' NIH grant entitled, "HHSN261201000023I TCGA-Breast Cancer".

The following grants are new to Dr. Marks's Active Support: Hwang's DoD grant entitled, "Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS"; Hwang's NIH grant, "5R01-CA185138-02"; Hwang's NIH grant, "5U10-CA180857-02"; Dr. Marks' Arizona State University grant entitled, "A Simple System for Early Detection of Breast Cancer".

**CURRENT:**

**Title:** Atlantic Breast and Gynecologic Clinical Validation Center
**Time Commitment:** 4.75 Calendar months
**Role:** P.I.
**Sponsor:** National Institutes of Health (5U01-CA084955-15)
**Contracting/Grants Officer:** Amy Knight, 301-846-6701, knighta@mail.nih.gov
**Dates:** 09/01/2010-6/30/2016
**Funding:** $309,819
**Goals/Specific Aims:** The goal of this project is to compare promising biomarkers for their utility in specific clinical applications for breast, ovarian and cervical cancer.
Aim 1: Testing markers to predict progression of pre-invasive breast disease (carcinoma in situ and benign proliferative lesions) to invasive breast cancer.
Aim 2: Comparison of auto-antibody panels for the detection of ovarian cancer.
Aim 3: Development of risk based decision modeling to incorporate common reproductive risk factors in screening algorithms for ovarian cancer.
Aim 4: Testing and implementation of markers to distinguish high risk cervical hyperplasias from low risk lesions.

**Title:** Atlantic Breast and Gynecologic Clinical Validation Center
**Time Commitment:** 1.0 Calendar month
**Role:** P.I.
**Sponsor:** National Institutes of Health (5U01-CA084955-14 Sub #2)
**Contracting/Grants Officer:** Amy Knight, 301-846-6701, knighta@mail.nih.gov
**Dates:** 09/01/2010-6/30/2016
**Funding:** $300,238
**Goals/Specific Aim:** The goal of this project is to compare promising biomarkers for their utility in specific clinical applications for breast, ovarian and cervical cancer.
Aim 1: Testing markers to predict progression of pre-invasive breast disease (carcinoma in situ and benign proliferative lesions) to invasive breast cancer.
Aim 2: Comparison of auto-antibody panels for the detection of ovarian cancer.

Aim 3: Development of risk based decision modeling to incorporate common reproductive risk factors in screening algorithms for ovarian cancer.
Aim 4: Testing and implementation of markers to distinguish high risk cervical hyperplasias from low risk lesions.

**Title:** Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS
**Time Commitment:** 2.40 calendar months
**Role:** Co-Investigator
**Sponsor:** Department of Defense, W81XWH-14-1-0473 (Hwang)
**Contracting Grants Officer:** Pending
**Dates:** 9/30/14-9/29/19
**Funding:** $1,482,598
**Goals/Specific Aims:** This proposal is based upon metapopulation and dispersal theories from ecology to predict cancer progression based on their effects on natural selection. This framework represents a novel approach that is a significant departure from traditional cancer biology, and could yield a universally applicable construct for understanding interactions between tumors and their environments.

**Title:** A Simple System for Early Detection of Breast Cancer
**Time Commitment:** 1.2 calendar
**Role:** PI
**Sponsor:** Arizona State University
**Contracting Grants Officer:** Unknown
**Dates:** 7/1/14-6/30/16
**Funding:** $119,484
**Goals/Specific Aims:** The goal of this project is to assemble a well-characterized cohort of serum and plasma from newly diagnosed breast cancer cases and matched controls for immunosignaturing. Assemble a cohort of newly diagnosed women with carcinoma in situ, atypical hyperplasia, and hyperplasia without atypia for immunosignaturing. Collect demographic and other exposure data on these cases and controls to allow for investigation into important co-variates for immunosignaturing.

**Title:** (PQC3) Genomic Diversity and Microenvironment as Drivers of Metastatis in DCIS
**Time Commitment:** 1.2 calendar months
**Role:** Co Investigator
**Sponsor:** National Institutes of Health, 5R01-CA185138-02 (Hwang)
**Contracting Grants Officer:** Suresh Mohla, 240-276-6220, mohlas@mail.nih.gov
**Dates:** 8/1/14-7/31/18
**Funding:** $337,027
**Goals/Specific Aims:** In this project, we propose to collect genomic, phenotypic, and radiographic measures of tumor cell diversity in DCIS and the tumor microenvironment, and test whether these diversity measures can identify which patients are most likely to develop metastatic disease. Deliverables from the proposal have high potential for rapid integration into clinical trials for active surveillance of DCIS, and in addition, could have universal relevance for management of other solid tumors.

**LO, JOSEPH, Ph.D.**

**Changes to Active Support:**
The following grants listed on Dr. Lo's previously submitted Active Support have ended: Lo's Siemens Medical Solutions grant entitled, "Collaboration between DUMC and SMS in the Field of Digital Mammography"; Segars' NIH grant "5R01-CA134658-04"; Baker's Siemens Medical Solutions grant entitled, "Multi Center Case Collection Study to Create a Library of Digital Mammography and Siemens Inspiration Digital Breast Tom".

The following grants are new to Dr. Lo's Active Support: Hwang's DoD grant entitled, "Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS"; Hwang's NIH grant, "5R01-CA185138-02".

**ACTIVE**

**Title:** Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS
**Time Commitment:** 1.20 calendar months
**Role:** Co Investigator
**Sponsor:** Department of Defense, W81XWH-14-1-0473 (Hwang)
**Contracting Grants Officer:** Pending
**Dates:** 9/30/14-9/29/19
**Funding:** $1,482,598
**Goals/Specific Aims:** This proposal is based upon metapopulation and dispersal theories from ecology to predict cancer progression based on their effects on natural selection. This framework represents a novel approach that is a significant departure from traditional cancer biology, and could yield a universally applicable construct for understanding interactions between tumors and their environments.

**Title:** (PQC3) Genomic Diversity and Microenvironment as Drivers of Metastatis in DCIS
**Time Commitment:** 1.2 calendar months
**Role:** Co Investigator
**Sponsor:** National Institutes of Health, 5R01-CA185138-02 (Hwang)
**Contracting Grants Officer:** Suresh Mohla, 240-276-6220, mohlas@mail.nih.gov
**Dates:** 8/1/14-7/31/18
**Funding:** $337,027
**Goals/Specific Aims:** In this project, we propose to collect genomic, phenotypic, and radiographic measures of tumor cell diversity in DCIS and the tumor microenvironment, and test whether these diversity measures can identify which patients are most likely to develop metastatic disease. Deliverables from the proposal have high potential for rapid integration into clinical trials for active surveillance of DCIS, and in addition, could have universal relevance for management of other solid tumors.

**BAKER, JAY, MD.**

**Changes to Active Support:**

The following grants listed on Dr. Baker's previously submitted Active Support have ended: Segars' NIH grant "5R01-CA134658-04"; Baker's Siemens Medical Solutions grant entitled, "Multi Center Case Collection Study to Create a Library of Digital Mammography and Siemens Inspiration Digital Breast Tom".

The following grants are new to Dr. Baker's Active Support: Hwang's DoD grant entitled, "Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS"; Hwang's NIH grant, "5R01-CA185138-02".

**ACTIVE**

**Title:** Genomic Diversity and the Microenvironment as Drivers of Progression in DCIS
**Time Commitment:** 0.60 calendar months
**Role:** Co Investigator
**Sponsor:** Department of Defense, W81XWH-14-1-0473 (Hwang)
**Contracting Grants Officer:** Pending
**Dates:** 9/30/14-9/29/19
**Funding:** $1,482,598
**Goals/Specific Aims:** This proposal is based upon metapopulation and dispersal theories from ecology to predict cancer progression based on their effects on natural selection. This framework represents a novel approach that is a significant departure from traditional cancer biology, and could yield a universally applicable construct for understanding interactions between tumors and their environments.

**Title:** (PQC3) Genomic Diversity and Microenvironment as Drivers of Metastatis in DCIS
**Time Commitment:** 0.87 calendar months
**Role:** Co Investigator
**Sponsor:** National Institutes of Health, 5R01-CA185138-02 (Hwang)
**Contracting Grants Officer:** Suresh Mohla, 240-276-6220, mohlas@mail.nih.gov
**Dates:** 8/1/14-7/31/18
**Funding:** $337,027
**Goals/Specific Aims:** In this project, we propose to collect genomic, phenotypic, and radiographic measures of tumor cell diversity in DCIS and the tumor microenvironment, and test whether these diversity measures can identify which patients are most likely to develop metastatic disease. Deliverables from the proposal have high potential for rapid integration into clinical trials for active surveillance of DCIS, and in addition, could have universal relevance for management of other solid tumors.

**ALLISON HALL, MD, PhD**

**Changes to Active Support:** Dr. Hall is new Key Personnel to this project; therefore, her full Other Support document is listed below

**ACTIVE SUPPORT**
**Title:** A Viable Solution for a See and Treat Paradigm for Cervical Pre-Cancer in Africa
**Time Commitment:** 0.6 calendar months

**Role:** Co-Investigator
**Sponsor:** NIH, 1R01-CA195500-01 (Ramanujam, Nirmala)
**Contracting Grants Officer:** Ted Williams, NCI 6120 Executive Blvd, Rockville, MD 20892
**Dates:** 7/1/15-6/30/18
**Funding:** $153,642
**Goals/Specific Aims:** The goal of this project is to leverage community-clinics as a way to bring early detection and treatment of cervical pre-cancer to as many patients as possible in LIMICs particularly in places where hospitals are not accessible.

**Title:** Culturally appropriate screening and diagnosis of cervical cancer in East Africa
**Time Commitment:** 0.12 calendar months
**Role:** Co Investigator
**Sponsor:** NIH 1R01-CA193380-01 (Ramanujam, Nirmala)
**Contracting Grants Officer:** Ted Williams, NCI 6120 Executive Blvd, Rockville, MD 20892
**Dates:** 8/1/15-7/31/20
**Funding:** $468,795
**Goals/Specific Aims:** The goal of this proposal is to develop a portable, low power consumption spectroscopic device that can be used to obtain accurate and reproducible quantitative measurements of absorption and scattering coefficients with application to screening of cervical cancers for global health.

**PENDING**
**Title:** Duke Breast Cancer Clinical Validation Center
**Time Commitment:** 2.4 calendar months
**Role:** Pathologist
**Sponsor:** NIH 2U01-CA084955-16 (Marks, Jeffrey)
**Contracting Grants Officer:** Lalita Palekar, NIC 6116 Executive Blvd, Rockville, MD 20892
**Dates:** 9/1/15-8/31/20
**Funding:** $509,957
**Goals/Specific Aims:** The goals/specific aim of this proposal is to create a Clinical Validation Center within the Early Detection Research Network that is focused on breast and gynecologic cancers.

**Title:** Vaccine Targeting Brachyury to Preven Therapy Resistant Breast Cancer
**Time Commitment:** 1.2 calendar months
**Role:** Co Investigator
**Sponsor:**NIH (Lyerly)
**Contracting Grants Officer:** Ted Williams, NCI 6120 Executive Blvd, Rockville, MD 20892
**Dates:** 12/1/15-11/30/18
**Funding:** $500,000
**Goals/Specific Aims:** The goal of this proposal is to test the hypothesis that destroying resistant cells as they emerge, while simultaneously inhibiting the growth of residual estrogen-sensitive cells, will prevent the progression of BC treated with endocrine therapies. The specific aim is to test this hypothesis in preclinical studies of endocrine therapy resistance and randomized phase II translational clinical trial administering anti-estrogen therapy (tamoxifen with or without a novel MVA-brachyury-TRICOM vaccine to patients with metastatic ER + breast cancer.

**Title:** A new methodology for investigating breast cancer intratumor heterogeneity jointly in MRI and pathology slides
**Time Commitment:** 1.2 calendar months
**Role:** Investigator
**Sponsor:** NIH (Mazurowski)
**Contracting Grants Officer:** Manzoor Zarger, CSR, RKL2-Two Rockledge Center, 6196, 6701 Rockledge Dr, Bethesda, MD 20892
**Dates:** 4/1/16-3/31/18
**Funding:** $150,000
**Goals/Specific Aims:** The goal of this project is to use previously collected gene expression data based on samples from at least two different subregions for each tumor to determine intratumor genomic heterogeneity and test whether quantitative, computer-extracted DCE-MRI feature can predict intratumor genomic heterogeneity. The specific aims are to develop a set of computer vision algorithms for segmentation and detailed analysis of tumors in breast DCE-MRI to extract a set of specific features potentially predictive of tumor genomic heterogeneity.

**PREVIOUS**
None

**OVERLAP**
None

- **What other organizations were involved as partners?**
    - **Organization Name:** University of California – San Francisco
    - **Location of Organization:** San Francisco, CA
    - **Partner's contribution to the project:** collaborating site, no longer active
        - **Financial support:** none
        - **In-kind support:** none
        - **Facilities:** no longer applicable
        - **Collaboration:** no longer applicable
        - **Personnel exchanges:** none
        - **Other:** none

    - **Organization Name:** Arizona State University (ASU)
    - **Location of Organization:** Phoenix, AZ
    - **Partner's contribution to the project:** Full collaborative site, Dr. Maley's current institution
        - **Financial support:** none
        - **In-kind support:** none
        - **Facilities:** Dr. Maley's laboratory and facilities
        - **Collaboration:** Entire project is built as a collaboration between Duke and ASU
        - **Personnel exchanges:** none
        - **Other:** none

- **Organization Name:** Washington University
- **Location of Organization:** St. Louis, MO
- **Partner's contribution to the project:** *(*Facilities & Collaboration) We are contracting with Wash. U. to provide the exome sequencing for our project. We are also informally collaborating with Dr. Elaine Mardis and her breast cancer team on this project.

## 8. SPECIAL REPORTING REQUIREMENTS

This is a collaborative award with Carlo Maley at Arizona State University.

## 9. APPENDICES

References:


1. Heindl, A., Nawaz, S. & Yuan, Y. Mapping spatial heterogeneity in the tumor microenvironment: a new era for digital pathology. *Lab Invest* **95**, 377-84 (2015).
2. Nawaz, S., Heindl, A., Koelble, K. & Yuan, Y. Beyond immune density: critical role of spatial heterogeneity in estrogen receptor-negative breast cancer. *Mod Pathol* **28**, 766-77 (2015).
3. Yuan, Y. Modelling the spatial heterogeneity and molecular correlates of lymphocytic infiltration in triple-negative breast cancer. *J R Soc Interface* **12** (2015).
4. Yuan, Y. et al. Quantitative image analysis of cellular heterogeneity in breast tumors complements genomic profiling. *Sci Transl Med* **4**, 157ra143 (2012).